Atopic dermatitis (AD) is a prevalent disease worldwide and is associated with systemic comorbidities representing a significant burden on patients, their families, and society. Therapeutic options for AD remain limited, in part because of a lack of well-characterized animal models. There has been increasing interest in developing experimental approaches to study the pathogenesis of human AD in vivo, in vitro, and in silico to better define pathophysiological mechanisms and identify novel therapeutic targets and biomarkers that predict therapeutic response. This review critically appraises a range of models, including genetic mutations relevant to AD, experimental challenge of human skin in vivo, tissue culture models, and human and computational models of atopic dermatitis.
integration of “omics” data sets, and development of predictive computational models. Although no one individual model recapitulates the complex AD pathophysiology, our review highlights insights gained into key elements of cutaneous biology, molecular pathways, and therapeutic target identification through each approach. Recent developments in computational analysis, including application of machine learning and a systems approach to data integration and predictive modeling, highlight the applicability of these methods to AD subclassification (endotyping), therapy development, and precision medicine. Such predictive modeling will highlight knowledge gaps, further inform refinement of biological models, and support new experimental and systems approaches to AD. (J Allergy Clin Immunol 2019;143:36-45.)

Key words: Atopic dermatitis, atopic eczema, endotype, human models, machine learning, mechanistic models, precision medicine, tissue culture models, skin equivalents, systems biology

Atopic dermatitis (AD; synonym atopic eczema) has a complex etiology involving multiple genetic and environmental factors. With its very high incidence in childhood, chronicity, devastating effect on quality of life for affected patients and their families, enormous socioeconomic costs, and limited therapeutic options to date, AD represents a major challenge. Furthermore, there is clear evidence that AD represents a systemic inflammatory disease with multiple comorbidities extending beyond the well-recognized atopic associations. Consequently, a number of animal models have been developed and used by investigators and the pharmaceutical industry to better understand the disease and consider new pathways to target. However, as recently reviewed, mouse models do not adequately reflect the transcriptomic and gene pathways activated in human AD skin, and the intrinsic difference between mouse and human skin represents a barrier to direct translation of findings from animals into human disease. Consequently, there has been increasing interest in experimental studies in human subjects (in part facilitated by technological and “omics” developments), cell culture models using human tissue, and use of computational or mathematical models that are developed by integrating these data.

In this review article we have used the term human AD model to define representations of the disease state and interventions that enable scientific insight into disease pathogenesis, disease course, and response to therapy. We delineate and critically appraise these AD modeling approaches that range from experimental study of human skin in vivo (including challenge studies and detailed phenotyping and investigation of patients harboring specific genetic mutations) to generation of AD-relevant models by using immunologic, genetic, and molecular methods in 2-dimensional and 3-dimensional human tissue culture to development of in silico computational models using a systems biology approach. Although by definition a reductionist approach cannot recapitulate the full spectrum of AD, these models have greatly increased our understanding of the molecular drivers of AD and provide a powerful tool for preclinical drug development and target validation. However, just as the etiology, clinical expression, and severity of AD range broadly among patients, in vitro and in silico models of AD vary widely both in how the AD phenotype is induced and how the models are evaluated. Therefore we invited members of the International Eczema Council (www.eczemacouncil.org), a group of experts in AD, and associated authorities in the field to contribute to a scoping and development meeting and subsequently to evaluate and critically appraise the breadth of human AD and computational models to determine their strengths and weaknesses in how they recapitulate the pathophysiology of AD and enable therapeutics to be tested and validated.

IN VIVO MODELS OF AD

Two general approaches using human in vivo models have been followed to dissect the pathogenesis of AD: (1) the study of rare genetic variants with AD-like phenotypes and (2) the experimental challenge of patients with or without AD with allergens or irritants. Regarding the first approach, numerous studies have characterized genetic disorders that display skin barrier function abnormalities. Most often, these studies characterized ichthyosis vulgaris, a disease that allowed insight into the function of the epidermal differentiation gene filaggrin (FLG), in which mutations show the strongest association to AD development of all known genes (Fig 1). Other studies have focused on disorders characterized by systemic inflammation and immunodeficiency with AD-like skin manifestations (Fig 1). One example is immunodyregulation, polyendocrinopathy, enteropathy, X-linked syndrome, which serves as a model to study how systemic imbalances in the regulatory T-cell population can drive cutaneous AD-like inflammation. In addition, the link between type 2 immunity; transcription factors, such as Janus kinase or signal transducer and activator of transcription; and high levels of IgE was investigated in immunodeficiency syndromes, such as signal transducer and activator of transcription 3 and dedicator of cytokinesis 8 hyper-IgE syndromes or combined immunodeficiency disorders.

Table E1 in this article’s Online Repository at www.jacionline.org lists the main genetic conditions that have provided insight into AD pathogenesis to date. Although the study of rare variants offers the opportunity to delineate distinct molecular mechanisms and control pathways of a particular phenotype and thus can be regarded as “human models of AD,” a limitation of this approach is that not all observed phenomena are relevant in patients with AD, which is more complex and heterogeneous than monogenic disorders.

The second in vivo approach to study the pathogenesis of AD is standardized challenge with allergens or other environmental factors. The most commonly used model is the atopy patch test (APT), an epicutaneous challenge of specific allergens dissolved in vehicle, which has provided insight into the temporal development of immune phenomena in patients with AD (see Table E2 in this article’s Online Repository at www.jacionline.org). Although developed in part to define clinically relevant reactions to aeroallergens, food allergens, and autoantigens, its validity and predictive value depend on a variety of factors.

Abbreviations used
ACD: Allergic contact dermatitis
AD: Atopic dermatitis
APT: Atopy patch test
FLG: Gene encoding filaggrin
LC: Langerhans cell

in the protocol, and the APT is not used routinely in clinical practice. Experimentally, the APT has provided insight into the temporal sequence of cutaneous cellular infiltrates. Acute skin lesions show a highly reproducible TH2-dominant infiltrate, although other cell types, including TH17 cells, are also present. This TH2 dominance is in sharp contrast to other inflammatory skin diseases, such as psoriasis. Time-course studies have shown that additional immune cell subsets, such as TH1 and TH22 cells, progressively infiltrate the skin during an ongoing APT reaction, mirroring the cellular composition of acute versus chronic human AD. The APT has also been used to characterize dendritic cells within early lesional AD skin, such as inflammatory dendritic epidermal cells. Furthermore, the APT has provided insight into the interaction of microbiota and our immune system, in particular the role of bacteria-derived superantigens acting as an amplifier of the allergen-specific cutaneous response in patients with AD.

All current challenge models have some limitations because they only represent acute reactions, and the small areas of application cannot reproduce the intense pruritus and sleep disturbances usually present in patients with AD. Furthermore, to date, they have not stratified for genetic differences/endotypes among patients with AD. For example, comparing APTs in patients with and without FLG mutations might be a useful line of future investigation. Moreover, in the future, molecular profiling of lesional skin from standardized challenge models adjusted according to AD endotype can be used in early clinical studies to evaluate the potential of new drugs to improve AD.

**IN VITRO MODELS**

As shown in Table E3 in this article’s Online Repository at www.jacionline.org, there are several 2-dimensional cell-culture and 3-dimensional organotypic models for AD that complement each other in addressing specific experimental questions. Although 2-dimensional cell culture models (by definition) do not duplicate the architecture of skin, they are amenable to high-throughput techniques for drug discovery and target validation (2-dimensional model section, see Table E3). Accordingly, Otsuka et al used 2-dimensional cultures to screen a chemical library for compounds that enhance FLG transcriptional activation and mRNA expression, suggesting a...
potential novel therapeutic agent for AD. On the other hand, 3-dimensional models replicate the stratified squamous epithelium of epidermis but require specific expertise and are time consuming. Epidermal equivalents consist of keratinocytes without a dermal compartment, whereas skin equivalents have a dermis, such as fibroblast-embedded collagen (3-dimensional model section, see Table E3). Both 2-dimensional and 3-dimensional models are amenable to treatment with disease-relevant cytokines, gene knockdown, use of patient-derived cells, and/or coculture (Fig 2 and see Table E3).

The immune system is a major driver of AD, and in vitro immune modulation with disease-relevant cytokines, such as interleukins, can lead to AD-like phenotypes in normal primary keratinocytes and 3-dimensional models (3-dimensional cytokine model section, see Table E3). Knockdown of filaggrin (FLG) in culture systems can provide insight into the molecular and proteomic changes associated with its loss in patients with AD, and combining FLG knockdown with other perturbations, such as cytokine treatment, can be used to study the multifactorial drivers of AD. For example, Honzke et al reported that FLG knockdown exacerbated epidermal responses to IL-4 and IL-13, including increased proliferation and keratinocyte-released cytokines in 3-dimensional skin equivalents. Patient-derived cells for 2-dimensional and 3-dimensional culture or tissue for explant culture are limited by access and availability but might be the most relevant in terms of modeling AD.

Furthermore, patients’ biopsy specimens can be a source of skin cells other than keratinocytes, allowing for coculture models. Given that multiple systems contribute to AD, coculture models that include immune cells, dermal fibroblasts, and neurons can begin to address their interplay with keratinocytes. For example, Berroth et al derived keratinocytes and fibroblasts from normal and AD skin and showed that AD-derived fibroblasts are sufficient to decrease FLG mRNA in normal-derived keratinocytes in 3-dimensional culture. Moreover, combining FLG knockdown with CD4+ activated T cells uncovered direct cross-talk between keratinocytes and T cells that resulted in T-cell migration within the dermal compartment toward the epidermis.

These studies highlight the levels of complexity that can be engineered into the 3-dimensional culture models. Three-dimensional culture systems have also been used to understand environmental influences on skin, including air pollution, UV radiation exposure, and bacterial infection. Therefore these relevant environmental factors could be incorporated into in vitro models of AD. The 3-dimensional cultures and skin explants can also be used to assess the comparative efficacy and practical applicability of novel drug delivery systems. Notably, despite the assorted methodologies applied in developing in vitro models of AD, there is overlap in the AD-like characteristics among the various models: most produce perturbed epidermal morphology, abnormal differentiation, and barrier dysfunction. Most often, disparities in reported phenotypes appear to stem, at least in part, from differences in the methodologies used in evaluating models (not necessarily because of the absence of the phenotype).

Although in vitro models might not mimic certain symptomatic and/or subjective aspects of the disease, such as pruritus and pain, they allow monitoring of changes in epidermal morphology and differentiation, gene and protein expression, lipid synthesis, and barrier function. Histologically, AD skin sections and most 3-dimensional models of AD show profound changes in the
epidermal compartment, including hypogranulosis, spongiosis, and increased cellularity because of hyperproliferation (3-dimensional model section, see Table E3). Changes in expression of genes (detected by using microarray, RNA-sequencing, or quantitative PCR) and protein (detected by using liquid chromatography–mass spectrometry, Western blotting, ELISA, or immunohistochemistry) can be used to evaluate disturbances in differentiation and immune response in 2-dimensional and 3-dimensional models. Lipid synthesis, which is required for optimal barrier function, can be monitored by expression of related enzymes or directly by using mass spectrometry. Epidermal barrier function can be monitored in 2-dimensional and 3-dimensional models, depending on the assay.

We recommend that the phenotype of any AD in vitro model should be extensively characterized and should include parallel analysis of epidermal morphology, differentiation status, loss or gain of key transcripts/proteins, analysis of immune components, and assessment of functional epidermal barrier parameters. Full characterization of any AD model can inform downstream evaluation of potential therapeutic agents with respect to reversing different aspects of the disease. Testing potential targets or drugs in several model types can add rigor and indicate whether a signaling pathway or protein is central to the diverse manifestations of AD.

**IN SILICO COMPUTATIONAL MODELS**

A core element of a systems biology approach is development of in silico computational models (mechanistic models) by means of integration of different types of experimental and clinical data from multiple studies, including those associated with disease conditions. In silico experiments (ie, computer simulations or mathematical analysis of in silico models) can test model-specific hypotheses, predict disease prognosis or treatment outcomes, and identify knowledge gaps, guiding future experiments and clinical trials that produce further data. This iterative process refines in silico models, providing holistic systems-level mechanistic insight into how perturbations (treatments or risk factors) lead to whole-organism phenotypes.

A mechanistic model describes causative interactions between the system’s components involved in the phenomena of interest (eg, disease or treatment outcomes). Existing mechanistic models of AD vary widely, depending on the levels of interaction (tissue, cells, proteins, and genes) included in the model and mathematical methods used to describe the interactions.

Dominguez-Hüttinger et al\(^\text{54}\) developed a multi-scale deterministic model that delineates interactions between the environment, skin barrier integrity, and immune activation using ordinary differential equations (Table I).\(^\text{54,51}\) Two bistable “switches” are described: the first regulating the onset of AD flares and the second controlling progression to severe and persistent disease. The model predicts, for example, that genetic predisposition to barrier dysfunction (eg, FLG haploinsufficiency) predisposes to longer flares and more persistent disease and that prophylactic emollient use might be beneficial (Table I).

For example, this computational model supports the need for a greater topical steroid treatment dose after disease worsening and the potential need for more frequent than 2 to 3 days per week application of topical steroid treatment to maintain remission\(^\text{52}\) in patients with FLG haploinsufficiency (Table I), presenting a readily testable stratification treatment regimen based on genotype.

Polak et al\(^\text{50}\) developed a stochastic Petri net model that delineates genetic regulatory mechanisms responsible for immune responses in Langerhans cells (LCs; Table I). The model describes reported interactions between interferon regulatory factors, interferon regulatory factor transcription partners, and DNA sequences in a logic-based diagram. In vitro experiments validated model predictions that the ability of LCs to present a peptide is altered by cytokine milieu and that a phosphoinositide 3-kinase γ inhibitor reduces the ability of LCs to induce T\(_{\text{H}}\)1 responses. These smaller-scale and focused mechanistic models can describe detailed interactions that are difficult to include and validate in multiscale models. Inclusion of the detailed interactions would make the multiscale models too complex to interpret and validate because of the current lack of quantitative dynamic data measuring the variables across different scales simultaneously.

Subramanian et al\(^\text{57}\) used a pathway model that included manually curated skin-specific pathways and relevant genes (Table I). Pathway enrichment analysis using transcriptomic data sets of patients with AD provided mechanistic insights into drug actions of topical betamethasone and pimecrolimus. The pathway model would allow in silico experiments once the kinetics parameters for pathways are identified to provide quantitative and dynamic predictions of disease progression and treatment outcomes.

Population pharmacokinetic and pharmacodynamic models have also been developed to describe differences and variability in pharmacologic effects observed in large clinical studies for AD treatment.\(^\text{58,59}\) The authors identified the model parameters that best fit the effects of nemolizumab and dupilumab measured in terms of AD severity score or pharmacokinetics (Table I).\(^\text{58,59}\) Population pharmacokinetic and pharmacodynamic models could help achieve mechanistic understanding of pharmacologic effects if combined with mechanistic models.

One of the challenges in developing mechanistic models is identification of the components and pathways relevant to the model-specific hypothesis to be tested. This can be achieved by using unbiased multivariate analyses of a collection of large-scale data, for example by using machine learning data analysis. Application of machine learning methods to AD-related data is relatively limited at present, but some relevant works have been already published. Thijs et al\(^\text{10}\) developed a piecewise linear mixed model to predict AD severity scores after different treatments, and Kiiski et al\(^\text{11}\) developed a multivariate logistic regression model to predict a “good treatment response.” A sufficient level of cross-validation is crucial to reduce bias and ensure the general applicability of models with predictive power beyond mere data description.

All the models presented above were developed based on the published data derived from studies in which the inclusion and exclusion criteria for AD were specified. Although the majority of studies used the Hanfin and Rajka criteria and specified further clinical (including comorbidities) and demographic details, it is clear that patients with AD present with a wide spectrum of
<table>
<thead>
<tr>
<th>Model type</th>
<th>Scientific merits</th>
<th>Clinical utility</th>
<th>Limitations</th>
<th>Key features</th>
<th>Key findings/predictions</th>
<th>References</th>
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<tbody>
<tr>
<td>Multiscale mechanistic model</td>
<td>Mechanistic understanding of system-level effects of potential triggers and processes on disease state</td>
<td>Identification of therapeutic targets and their mechanisms for further clinical investigation</td>
<td>Prediction of dynamic effects of therapeutics, leading to patient stratification</td>
<td>Models developed based on hypothesized relationships that were previously described experimentally</td>
<td>A hybrid ordinary differential equation model of the dynamic interplay between skin barrier function, immune responses, and environmental stressors that determines AD pathogenesis</td>
<td>Preventive effects of emollients against AD progression (shown by clinical trials) Synergistic effects of environmental (eg, microbiome) and genetic (eg, FLG) risk factors on AD progression (shown by mouse experiments with ovalbumin challenge or dose-dependent effects of FLG deficiency) Poor adherence to the suggested optimal treatment schedule leads to higher treatment doses. Application of corticosteroids for 2 consecutive days per week is optimal for the maintenance period.</td>
</tr>
<tr>
<td>Gene regulatory network model</td>
<td>Understanding of gene regulatory mechanisms behind disease processes</td>
<td>Identification of therapeutic targets and their mechanisms at the gene regulation level</td>
<td>Models developed based on published genetic interactions</td>
<td>Stochastic Petri net model of interferon regulatory factor gene regulatory network in response to \textit{in vitro} treatment of LCs with TNF-(\alpha) and TSLP</td>
<td>In \textit{vitro} experiments validated predictions that the ability of LCs to present a peptide is altered by cytokine milieu and that PI3K(\gamma) inhibitor reduces the ability of LCs to induce T(\text{H}1) responses.</td>
<td>Polak et al (^5)</td>
</tr>
<tr>
<td>Pathway models</td>
<td>Understanding of disease mechanisms</td>
<td>Identification of therapeutic targets and their mechanisms</td>
<td>Models developed based on published pathways</td>
<td>A pathway model including 35 manually curated skin-specific pathways and &gt;2600 genes</td>
<td>Pathway enrichment analysis using transcriptomic data sets of 10 patients with AD treated with betamethasone valerate and pimecrolimus predicted mechanism of action of both drugs on human skin.</td>
<td>Subramanian et al (^5)</td>
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clinical and molecular features (including, for example, a greater heterogeneity in transcriptomic profile of lesional skin compared with psoriasis).  

**FUTURE DEVELOPMENTS**

The development of more sophisticated human and computational models of AD that integrate large-scale clinical and “omics” data offer the potential for a deeper understanding of disease endotypes, molecular mechanisms underlying key pathogenic events and clinical hallmarks of AD, as well as prediction of therapeutic outcomes, including comorbidity at the level of an individual patient. Accepting that, by definition, these models are based on a reductionist approach, they need to reflect the complexity of AD pathogenesis, including epidermal barrier dysfunction, altered penetration of chemicals and allergens, host/environment interaction, type 2 immunity, and tissue remodeling. We have illustrated in this review that the main approaches available today are in vitro models, identification and characterization of human inherited syndromes resembling AD, in vivo challenges of patients with AD, and in silico models. Here we speculate how the future of AD research will likely inform the development of more refined models of AD.

Refinement is likely to depend, at least in part, on methodological advances in the field and the additional information generated by novel approaches. For example, single-cell sequencing has recently identified novel rare but important immunologic subsets, and intravital photon microscopy has enabled visualization of cell-cell communication during inflammation. Application of this technology to AD is likely to inform the inclusion of distinct epithelial and immune cell types, and/or genetically modified primary human cells. Furthermore, small-scale spheroid organoids can enhance high-throughput approaches in the field. Finally, we expect that a technological breakthrough in the development of 3-dimensional skin models will be facilitated by cell printers.

### TABLE I. (Continued)

<table>
<thead>
<tr>
<th>Model type</th>
<th>Scientific merits</th>
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</tr>
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<tbody>
<tr>
<td>Population PK/PD models</td>
<td>Understanding of differences and variability in pharmacologic effects among a target population from clinical trial data</td>
<td>Prediction of optimal dose regimen</td>
<td>Requires large clinical data to have sufficient predictive power</td>
<td>PK/PD model for serum nemolizumab and pruritus VAS developed from 299 patients’ time course data</td>
<td>An appropriate flat dose regimen that is independent of body weights is used.</td>
<td>Saito et al</td>
</tr>
<tr>
<td>Machine learning predictive models</td>
<td>Unbiased analyses of differences between disease and nondisease (including treated tissue/patients and prediction of clinical outcomes (prognostic and therapeutic)</td>
<td>Identification of disease and therapeutic targets</td>
<td>Causative mechanisms remain largely unknown</td>
<td>Piecewise linear mixed models to predict EASI scores at 3 future time points from baseline biomarkers Developed from data of 150 serum biomarkers measured in 193 patients with AD</td>
<td>Combination of TARC, IL-22, and sIL-2R provides a good predictor for future EASI score.</td>
<td>Thijs et al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Findings can feed into mechanistic models</td>
<td>Machine learning applications to atopic eczema relatively limited at present</td>
<td>Multivariate logistic regression model to identify predictors of long-term response to topical maintenance treatment in AD on 169 patients</td>
<td>Serum total IgE (rather than the initial severity) is the most important factor predicting a good long-term treatment outcome.</td>
<td>Kiiski et al</td>
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</table>

*EASI, Eczema Area and Severity Index; IL-4Ra, IL-4 receptor antagonist; PI3Kγ, phosphoinositide 3-kinase γ; PK/PD, pharmacokinetics/pharmacodynamics; sIL-2R, soluble IL-2 receptor; VAS, visual analog scale.*
Deep neural networks are being applied as artificial intelligence tools to facilitate physician interpretation in the field of melanoma diagnostics and increasingly as methods to enable large data set integration. The first examples of disease classifiers and prediction of disease severity from biomarker sets have recently been published, and we expect this line of development to continue while ensuring a sufficient level of validation. We anticipate that refinement of these methods, in combination with in silico models, can lead to computational approaches and predictive models applied to diagnostics and therapeutic stratification.

The descriptive disease ontology of inflammatory skin diseases will need to be revised by shifting to pathogenesis-oriented structure and, in the future, by better definition of disease endotypes based on integration of multiomics data, clinical features, and clinical response to therapy in light of in silico models as assessed in large-scale and longitudinal cohorts. These advances are likely to inform the development of many of the current models.

However, to achieve a substantial breakthrough, we expect that different approaches will need to be combined, integrated, standardized, and performed at larger scale (Fig 3). For example, observations made in rare human disease variants or through specific challenge models in patients with AD can be validated in vitro and mapped to disease signatures in silico. Validation of functional hypotheses will increasingly depend on cross-referencing of data derived from clinical samples with outputs from in vitro models. Integration of clinical, biomarker, pharmacokinetic and pharmacodynamic (topical and/or systemic), and clinical outcome data will inform therapy development and precision medicine. Notably, all of our models depend on how precisely a particular question is asked and the quality of the clinical input, including the clinical metadata and integration with “omics” data derived from clinical samples. Finally, advanced statistical and machine learning analysis combined with in silico predictive modeling will be required to integrate information throughout all described layers and data sets to elucidate underlying mechanisms (and endotypes), further highlighting the importance of data standardization and scientific networking.

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REFERENCES


